

*AMENDMENTS TO THE CLAIMS*

This listing of claims replaces all prior versions, and listings, of claims in the application.

1-8. (Canceled).

9. (Currently Amended) A method for real-time detecting and quantifying a first ~~nucleic acid template~~ amplicon and a second amplicon ~~nucleic acid template~~ in a PCR mixture comprising the steps of

- a) thermally cycling a PCR ~~mixture~~ mixture, wherein the PCR mixture comprises a thermostable polymerase, a double stranded DNA intercalating dye, the first template and the second template, primers for amplifying a first amplicon from the first template and a second amplicon from the second template, and wherein the first amplicon has a first  $T_m$  and the second amplicon has a second  $T_m$  and the first  $T_m$  is less than the second  $T_m$ ;
- b) obtaining during each thermal cycle ~~by cycle~~ a first emission reading of the double stranded DNA intercalating dye at a first measuring ~~temperature~~ temperature, wherein the first measuring temperature is between an annealing/extension temperature and the first  $T_m$ , first  $T_m$  and a second emission reading of the double stranded DNA intercalating dye at a second measuring ~~temperature~~ temperature, wherein the second measuring temperature is between the first  $T_m$  and the second  $T_m$ ;
- c) determining during each thermal cycle ~~by cycle~~ a first emission amount of the first amplicon which is the difference between the first emission reading and the second emission reading, and a second emission amount of the second amplicon which is the second emission reading.

10. (Canceled).

11. (Canceled).

12. (Currently Amended) The method of claim 9, ~~claim 11~~ wherein the double stranded DNA intercalating dye is selected from the group consisting of ethidium bromide, YO-PRO-1, Hoechst 33258, SYBR Gold, and SYBR Green I.

13. (Canceled).

14. (Canceled).

15. (Currently Amended) The method of claim 9, ~~claim 9~~ wherein the first measuring temperature is 0.25°C below the first  $T_m$ , 0.5°C below the first  $T_m$ , 1.0°C below the first  $T_m$ , 1.5°C below the first  $T_m$ , or 2.0°C below the first  $T_m$ , and wherein the first measuring temperature is higher than the annealing temperature.

16. (Currently Amended) The method of claim 9, ~~claim 9~~ wherein the second measuring temperature is 0.25°C below the second  $T_m$ , 0.5°C below the second  $T_m$ , 1.0°C below the second  $T_m$ , 1.5°C below the second  $T_m$ , or 2.0°C below the second  $T_m$ , and wherein the second measuring temperature is higher than the first  $T_m$ .

17. (Currently Amended) The method of claim 9, ~~claim 9~~ wherein the second measuring temperature is 0.25°C above the first  $T_m$ , 0.5°C above the first  $T_m$ , 1.0°C above the first  $T_m$ , 1.5°C above the first  $T_m$ , or 2.0°C above the first  $T_m$ , and wherein the second measuring temperature is less than the second  $T_m$ .

18. (Currently Amended) The method of claim 9, ~~claim 9~~ wherein the second measuring temperature is the first  $T_m + 0.25^\circ\text{C} < \text{the second measuring temperature} < \text{the second } T_m - 0.25^\circ\text{C}$ , the first  $T_m + 0.5^\circ\text{C} < \text{the second measuring temperature} < \text{the second } T_m - 0.5^\circ\text{C}$ , the first  $T_m + 1.0^\circ\text{C} < \text{the second measuring temperature} < \text{the second } T_m - 1.0^\circ\text{C}$ , the first  $T_m + 1.5^\circ\text{C} < \text{the second measuring temperature} < \text{the second } T_m - 1.5^\circ\text{C}$ , or the first  $T_m + 2.0^\circ\text{C} < \text{the second measuring temperature} < \text{the second } T_m - 2.0^\circ\text{C}$ .

19. (Canceled).

20. (Currently Amended) The method of claim 9, ~~claim 9~~ wherein the first emission amount of the first amplicon is obtained through a computer program performing a calculation of subtracting the first emission reading from the second emission reading or subtracting the second emission reading from the first emission reading.

21. (Currently Amended) A method for real-time detecting and quantifying a first ~~nucleic acid template~~ amplicon and a second amplicon ~~nucleic acid template~~ in a PCR mixture comprising the steps of:

- a) thermally cycling a PCR ~~mixture~~ mixture, wherein the PCR mixture comprises a thermostable polymerase, a double stranded DNA intercalating dye, the first template and the second template, primers for amplifying a first amplicon from the first template and a second amplicon from the second template, and wherein the first amplicon has a first  $T_m$  and the second amplicon has a second  $T_m$  and the first  $T_m$  is less than the second  $T_m$ ;
- b) obtaining during each thermal cycle ~~by cycle~~ a first pre- $T_m$  emission reading of the double stranded DNA intercalating dye at a measuring temperature which is below the first  $T_m$  and a first post- $T_m$  emission reading of the double stranded DNA intercalating dye at a ~~at the~~ measuring temperature which is above the first  $T_m$  and a second pre- $T_m$  emission reading of the double stranded DNA intercalating dye at a measuring temperature which is below the second  $T_m$  and a second post- $T_m$  emission reading of the double stranded DNA intercalating dye at the a measuring temperature which is above the second  $T_m$ ;
- c) determining during each thermal cycle ~~by cycle~~ a first emission amount of the first amplicon which is the difference between the first pre- $T_m$  emission reading and the first post- $T_m$  emission reading; and a second emission amount of the second amplicon which is the difference between the second pre- $T_m$  emission reading and the second post- $T_m$  emission reading.

22. (Canceled).

23. (Currently Amended) The method of claim 21, ~~claim 22~~ wherein the double stranded DNA intercalating dye is selected from the group consisting of ethidium bromide, YO-PRO-1, Hoechst 33258, SYBR Gold, and SYBR Green I.

24. (Canceled).

25. (Canceled).

26. (Currently Amended) The method of claim 21, ~~claim 21~~ wherein the measuring temperature below the first  $T_m$  and/or the second  $T_m$  are 0.25°C below, 0.5°C below, 1.0°C below, 1.5°C below, or 2.0°C below.

27. (Currently Amended) The method of claim 21, ~~claim 21~~ wherein the measuring temperature above the first  $T_m$  and/or the second  $T_m$  are 0.25°C above, 0.5°C above, 1.0°C above, 1.5°C above, or 2.0°C above.

28. (Currently Amended) The method of claim 21, ~~claim 21~~ wherein the first emission amount of the first amplicon is obtained through a computer program performing the calculation of subtracting the first pre- $T_m$  emission reading from the first post- $T_m$  emission reading or subtracting the first post- $T_m$  emission reading from the first pre- $T_m$  emission reading, and the second emission amount of the second amplicon is obtained through the computer program performing the calculation of subtracting the second pre- $T_m$  emission reading from the second post- $T_m$  emission reading or subtracting the second post- $T_m$  emission reading from the second pre- $T_m$  emission reading.

29-84. (Canceled).

85. (Currently Amended) A method for real-time detecting and quantifying a first ~~nucleic acid template~~ amplicon and a second amplicon ~~nucleic acid template~~ in a PCR mixture comprising the steps of

- a) thermally cycling a PCR mixture, ~~mixture~~ wherein the PCR mixture comprises a thermostable polymerase, a double stranded DNA intercalating dye, the first template and the second template, primers for amplifying a first amplicon from the first template and a second amplicon from the second template, and wherein the first amplicon has a first  $T_m$  and the second amplicon has a second  $T_m$  and the first  $T_m$  is less than the second  $T_m$ ;
- b) obtaining during each thermal cycle ~~by cycle~~ a first emission reading of the double stranded DNA intercalating dye at a first measuring temperature, wherein the first measuring temperature is between an annealing/extension

temperature and the first  $T_m$ , a second emission reading of the double stranded DNA intercalating dye at a second measuring temperature, wherein the second measuring temperature is between the first  $T_m$  and the second  $T_m$ ; and a third emission reading of the double stranded DNA intercalating dye at a third measuring temperature, wherein the third measuring temperature is between the second  $T_m$  and a total denaturing temperature; and

c) determining during each thermal cycle ~~by eye~~ a first emission amount of the first amplicon which is the difference between the first emission reading and the second emission reading, and a second emission amount of the second amplicon which is the difference between the second emission reading and the third emission reading.

86. (Canceled).

87. (Currently Amended) The method of claim 85, ~~claim 86~~ wherein the double stranded DNA intercalating dye is selected from the group consisting of ethidium bromide, YO-PRO-1, Hoechst 33258, SYBR Gold, and SYBR Green I.

88. (Canceled).

89. (Canceled).

90. (Currently Amended) The method of claim 85, ~~claim 85~~ wherein the first measuring temperature is 0.25°C below the first  $T_m$ , 0.5°C below the first  $T_m$ , 1.0°C below the first  $T_m$ , 1.5°C below the first  $T_m$ , or 2.0°C below the first  $T_m$ , and wherein the first measuring temperature is higher than the annealing temperature.

91. (Currently Amended) The method of claim 85, ~~claim 85~~ wherein the second measuring temperature is 0.25°C below the second  $T_m$ , 0.5°C below the second  $T_m$ , 1.0°C below the second  $T_m$ , 1.5°C below the second  $T_m$ , or 2.0°C below the second  $T_m$ , and wherein the second measuring temperature is higher than the first  $T_m$ .

92. (Currently Amended) The method of claim 85, ~~claim 85~~ wherein the second measuring temperature is 0.25°C above the first  $T_m$ , 0.5°C above the first  $T_m$ , 1.0°C above the

first  $T_m$ ,  $1.5^{\circ}\text{C}$  above the first  $T_m$ , or  $2.0^{\circ}\text{C}$  above the first  $T_m$ , and wherein the second measuring temperature is less than the second  $T_m$ .

93. (Currently Amended) The method of claim 85, ~~claim 85~~ wherein the second measuring temperature is the first  $T_m + 0.25^{\circ}\text{C} < \text{the second measuring temperature} < \text{the second } T_m - 0.25^{\circ}\text{C}$ , the first  $T_m + 0.5^{\circ}\text{C} < \text{the second measuring temperature} < \text{the second } T - 0.5^{\circ}\text{C}$ , the first  $T_m + 1.0^{\circ}\text{C} < \text{the second measuring temperature} < \text{the second } T_m - 1.0^{\circ}\text{C}$ , the first  $T_m + 1.5^{\circ}\text{C} < \text{the second measuring temperature} < \text{the second } T_m - 1.5^{\circ}\text{C}$ , or the first  $T_m + 2.0^{\circ}\text{C} < \text{the second measuring temperature} < \text{the second } T_m - 2.0^{\circ}\text{C}$ .

94. (Currently Amended) The method of claim 85, ~~claim 85~~ wherein the third measuring temperature is  $0.25^{\circ}\text{C}$  above the second  $T_m$ ,  $0.5^{\circ}\text{C}$  the second  $T_m$ ,  $1.0^{\circ}\text{C}$  above the second  $T_m$ ,  $1.5^{\circ}\text{C}$  above the second  $T_m$ , or  $2.0^{\circ}\text{C}$  above the second  $T_m$ , and wherein the third measuring temperature is less than the total denaturing temperature.

95. (Currently Amended) The method of claim 85, ~~claim 85~~ wherein the first emission amount of the first amplicon is obtained through a computer program performing a calculation of subtracting the first emission reading from the second emission reading or subtracting the second emission reading from the first emission reading, and the second emission amount of the second amplicon is obtained through a computer program performing a calculation of subtracting the second emission reading from the third emission reading or subtracting the third emission reading from the second emission reading.

96. (Currently Amended) The method of claim 21, ~~claim 21~~ wherein the measuring temperature above the first  $T_m$  and the measuring temperature below the second  $T_m$  ~~is the~~ are the same.